some potential in atmospheric oxygenate analysis although AED sensitivity for oxygen is only around 100 pg s^{-1}. Detectors such as the helium ionization detector (HID), which produce a nonselective high sensitivity response to these types of compounds, may in future allow on-line measurements of oxygenates with GC, assuming sufficient chromatographic resolution or trapping selectivity can be obtained.

Future Work

Gas chromatography has an important role to play in monitoring mankind's emissions into the atmosphere and exploring the natural balance of biogenically released materials. New developments in injection technology and adsorbent materials will allow a greater number of species to be determined automatically in field locations. Development in column technology to reduce the effect of moisture on chromatographic separation and broaden the range of volatilities that may be separated on a given column will also bring significant benefits. Improvements in detector sensitivities and reliability (notably bench-top MS) will determine which of the many available detectors become standard in the next generation of atmospheric instruments.

See Colour Plates 56, 57, 58.

See also: **II/Chromatography: Gas:** Detectors: General (Flame Ionization Detectors and Thermal Conductivity Detectors); Detectors: Mass Spectrometry; Detectors: Selective. **III/Environmental Applications:** Gas Chromatography-Mass Spectrometry.

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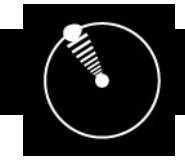
BACTERIOPHAGES: SEPARATION OF



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Introduction

Bacteriophages are viruses that infect bacteria. Historically, interest in bacteriophages was first generated by the possibility of using bacteriophages as biological antibiotics. This interest is periodically revived when difficulties are encountered with the use of chemical antibiotics. Interest in bacteriophages was also generated by both the short life cycle and the simple (short) genome of bacteriophages. These characteristics were useful in developing the science of molecular genetics. Bacteriophages were a favourite

for asking questions about transfer of biological information via DNA replication, transcription (copying of RNA from DNA) and translation (using the information in RNA to assemble proteins). Today, bacteriophages are used to carry small pieces of the DNA of higher organisms. The purpose is to determine the nucleotide sequence of the pieces and, ultimately, the nucleotide sequence of the whole genome of higher organisms. Basic science uses bacteriophages as models for understanding how separate molecules assemble to form a complex structure. The questions asked in these studies are fundamental: How do biological motors work? How is specificity maintained in intermolecular binding? How is accuracy assured during assembly? To what extent is biological form determined by either pathway of assembly or energetics of the final structure? Are errors corrected during assembly of biological macromolecules? Are separate biochemical processes integrated in the context of the interior of a cell? Pursuit of the answers to all these questions requires purification of bacteriophage particles.

Purification of both bacteriophages and bacteriophage-like particles is also needed for other types of study. Bacteriophage-like particles are present in both lakes and oceans. Some are true bacteriophages. Others are viruses that infect algae, rather than bacteria. Environmental biologists detect and sometimes purify these particles without knowing what they are. Purification helps establish what they are, both biologically and chemically. Finally, bacteriophages are model viruses. Study of the evolution of bacteriophages helps in the understanding of the evolution of other viruses, including pathogenic viruses. A major advantage of studying bacteriophages is their comparatively short life cycle. For example, bacteriophage T7 has a 13 min life cycle at 37°C and a 25 min life cycle at 30°C. The host cell is *Escherichia coli*. Most other bacteriophages that infect *E. coli* have life cycles less than 60 min at 37°C. Thus, studies of evolution are achieved more quickly than they are with any other organism. Also, the short life cycle of bacteriophages reduces the time needed to grow them.

Growth of Bacteriophages

Bacteriophages are grown in either gelled or liquid solutions of nutrients. Gels are made of the polysaccharide, agar. Gel-embedded infected cells are used to count viable bacteriophage particles. The procedure is to embed bacteriophages in the gel, together with host cells. The number of host cells is much greater than the number of bacteriophages. This gelled mixture is incubated to replicate the cells and infect them. A bacteriophage particle infects a growing host cell while in the gel. The cell bursts at the end of the infection. Light-scattering (turbidity) decreases when the infected cell bursts. All progeny of a single bacteriophage particle remain in a restricted region of the gel. This region is comparatively clear (nonturbid). The clear region is called a plaque. A viable bacteriophage particle is assayed by the formation of a single plaque on a glass or plastic Petri dish or plate which holds the gel with plaques. Plaques are counted after incubating viable bacteriophage particles with host cells. The number of viable bacteriophage particles per plate is small enough (usually 100–2000) so that overlapping plaques do not cause difficulty in counting. The number of plaques is multiplied by a dilution factor, if the bacteriophage particles had been diluted before assay. Bacteriophage plaques are illustrated in **Figure 1**.

Incubation of a host–bacteriophage mixture in a gel can also be used to prepare large numbers of bacteriophage particles before purification. The bacteriophages produced are sometimes called a plate stock. Preparing of bacteriophage particles via large volume (> 100 mL) plate stocks is not efficient (in time and cost), in comparison to preparing bacteriophage particles via infection in liquid culture. However, some bacteriophages which do not grow well in liquid cultures grow comparatively well when prepared as a plate stock. Thus, plate stocks are sometimes used for the growth of bacteriophage particles before purification. To remove bacteriophage particles from the gel of a plate stock the gel is minced

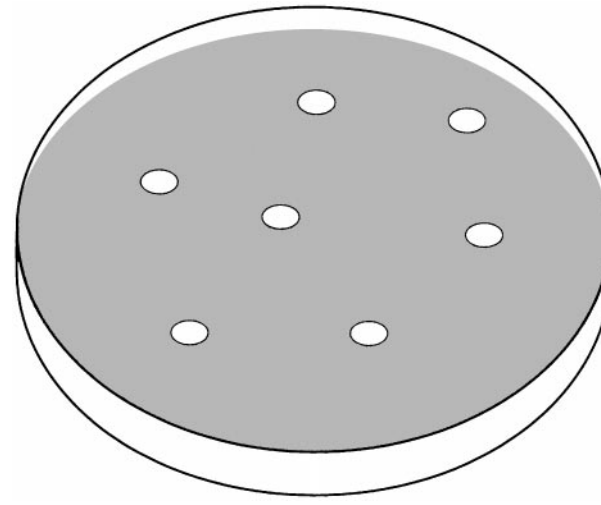

Figure 1 Plaques of bacteriophage T7. A Petri plate is filled with a 1% agar gel in an enriched growth medium. Subsequently, 0.7% molten agar in the same enriched medium is mixed with both host bacteria and bacteriophage particles. This mixture is spread on the 1.0% gel. The plate is incubated at 37°C. A plaque forms at the position of a single bacteriophage particle.

with either a glass rod or a spatula and the minced gel is incubated with buffer. The bacteriophage particles diffuse out of the gel, into the buffer during this process. Centrifugal pelleting is then used to remove the pieces of gel, together with pieces of the host bacterium. Some bacteriophages are pelleted with the gel. Thus, the pelleted pieces of gel are resuspended in buffer and then pelleted a second time. This process is called washing. Washing is typically performed two or three times. The supernatant solutions are pooled to produce a clarified plate stock.

Details of plate stock preparation vary slightly among the various bacteriophages. A bacteriophage best prepared by plate stock is bacteriophage G. Bacteriophage G is the largest bacteriophage (known to the author) that can be grown in culture. Bacteriophage G has a double-stranded DNA genome 670 kilobase pairs long. Bacteriophage G can be grown in liquid cultures, but results are erratic and greater success has been achieved with plate stocks. Typically, plate stocks of bacteriophage G are clarified by centrifugation at 5000 rpm in a 250 mL bottle (or the equivalent; centrifugal force at the bottom of the bottle is 3800 **g**). This speed is doubled (centrifugal force is quadrupled) in the case of smaller bacteriophages that don't sediment as quickly as bacteriophage G. Pelleting the bacteriophage particles (or aggregates of them) works against the objective of clarifying a plate stock.

Maintaining the stability of the bacteriophage particles is an objective at all stages of purification. Known bacteriophages are stabilized by the presence of divalent cations. Magnesium is usually used. Thus, magnesium should be present in the buffer. The presence of 0.001 mol L^{-1} $MgCl_2$ is usually sufficient for stability. Some salt (usually NaCl) should also be present. Some bacteriophages increasingly adsorb to fragments of host cell, as the salt concentration is lowered. Thus, salt concentrations are sometimes raised from the typical 0.1 mol L^{-1} to 0.5 mol L^{-1} or more. Adsorption to host cell debris can either inactivate a bacteriophage particle or cause it to pellet with the fragments of gel.

Storage

Plate stocks are also sometimes preferred when a new bacteriophage is isolated. The new bacteriophage might have been isolated from the wild. It might also have been produced by genetic modification of a previously isolated bacteriophage. A plate stock made with a single plate is a rapid and simple way of preserving this new strain. Preservation is completed by freezing a clarified plate stock. Freezing (typically at $-70^{\circ}C$) is used to prevent inactivation during storage for periods of years to decades. A cryoprotectant is added before freezing. Glycerol (10%) can be used as a cryoprotectant. Alternatively, a high molecular weight cryoprotectant can be used. A high molecular cryoprotectant sometimes used is 10% dextran, average molecular weight = 10 000. High molecular weight cryoprotectants are less likely to enter a bacteriophage particle. Therefore, high molecular weight cryoprotectants are less likely to cause bacteriophage particles to burst from osmotic shock during thawing. Osmotic shock during thawing occurs because freezing causes nonuniformity in the concentration of cryoprotectant. If the bacteriophage particle is in a region of comparatively low cryoprotectant concentration, an outward osmotic pressure gradient will develop. This is a demonstrated cause of inactivation of bacteriophage T4 by freeze–thawing.

Growth in Liquid Culture

A bacteriophage is usually grown in liquid culture, when the purpose is either chemical or physical characterization of either the bacteriophage or its nucleic acid. In this case, amounts in the 1–50 mg range may be needed. Either simple, well-defined media or enriched media are used. A simple, well-defined medium might have glucose as the primary (sometimes only) source of both carbon and energy. The medium is typically buffered with phosphate. The medium is supplemented with ammonium chloride to provide nitrogen for proteins. Other salts are also added. Both magnesium sulfate and calcium chloride are added after sterilization by autoclaving. Apparently, some requirements, such as iron, are present in sufficient quantity as contaminants. Alternatively, an enriched, but less well-defined, medium can be used. The major components of an enriched medium are often both tryptone and an extract of yeast cells.

Some bacteriophages are more easily (and more inexpensively) grown in minimal medium. This is true for some lytic double-stranded DNA bacteriophages, for example. A lytic bacteriophage always produces progeny when it infects a cell. The counterpart of a lytic bacteriophage is a lysogenic bacteriophage. A lysogenic bacteriophage may or may not produce progeny. The lysogenic bacteriophage genome both remains in and replicates with the host, if progeny are not produced. This state is called lysogeny. Lysogeny can simplify growth of some bacteriophages, as described below. Growth of a lytic bacteriophage, but not a lysogenic bacteriophage, encounters the following problem: cells are infected at a low ratio of bacteriophage particles to host cells, typically 0.01–0.1. Multiple cycles of infection occur. Therefore, the concentration of cells during the final (and most critical) cycle of infection is hard to control. Overgrowth of

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cells can result in a suboptimal yield, because of inadequate aeration. Undergrowth of cells can result in suboptimal yield, because of the low concentration of cells. The more rapidly cells grow, the more difficult controlling their concentration during the last cycle is. Cells grow more slowly in minimal medium (typical doubling time $= 1.5$–2.0 h for *E. coli* at 30°C) than they do in enriched medium (typical doubling time = 30–40 min for *E. coli* at 30°C). Thus, the infection is more easily controlled in minimal medium. None the less, other factors are also involved. Some experimentation with growth medium is required to optimize conditions of growth, when these conditions have not been previously optimized.

Growing lysogenic bacteriophages is usually easier than growing lytic bacteriophages. Lysogenic bacteriophages can be made to leave a state of lysogeny and enter a lytic cycle. Some mutants will do this when the temperature is raised. Thus, growth of lysogenic bacteriophages (bacteriophages λ and P22, for example) can be simplified. Host cells in a state of lysogeny are grown to an optimal concentration. The temperature is raised to induce the lytic cycle. The temperature is then lowered to an optimal temperature for growth. This process is useful for producing bacteriophage particles in large amount. It is also useful for producing other components of bacteriophage-infected cells. For example, some components of bacteriophage-infected cells retain their metabolic activity when infected cells burst (lyse is a frequently used synonym for burst). These activities include packaging of double-stranded DNA in bacteriophage capsids. Thus, a fragment of foreign DNA can be cloned by, first, incorporating the fragment in a bacteriophage genome, and, then, packaging the DNA *in vitro* by incubating the DNA in an extract of lysed, infected cells. Extracts of lysed, bacteriophage-infected cells can sometimes be used for incorporating the foreign DNA, as well as packaging it. An extract can be made by use of a lytic bacteriophage, as well as a lysogenic bacteriophage. The process is, however, less time- and resource-consuming with a lysogenic bacteriophage.

Equipment for Large–Scale Growth of Bacteriophages in Liquid Culture

Small scale (1–1000 mL) liquid cultures are grown in typical laboratory glassware. A flask is sometimes used, with aeration by shaking. Alternatively, a bottle is used with aeration via a bubbler and forced air. The latter alternative is simpler, less expensive and less space-consuming. A reliable shaker is needed in the former case; a source of forced air is needed in the latter. A reliable and reliably clean source of forced air is an aerator designed for use with tropical fish. The use of forced air is scalable to at least a 15 L culture. Shaking is scalable to roughly a 1 L culture.

The control of temperature can be achieved via several routes. A temperature-regulated fermentor with forced air can be used. The cost and inconvenience can, however, be dramatically lowered by using a bottle in a temperature-controlled water bath. Even a bottle in a temperature-controlled, water-filled sink can be used. In the latter two cases, a bottle with sterile medium and bubblers is aerated via forced air.

First Stage of Purification

The targeted degree of purification varies with the intent of the investigator. The minimal purification is removing large fragments of the host cell. Pelleting in a centrifuge is used. This stabilizes the bacteriophages by preventing adsorption of the bacteriophages to fragments of host cell membrane. Pelleting is typically done immediately after lysis for small (< 1 L) cultures. Pelleting is sometimes delayed until after precipitation of bacteriophage particles for large cultures. The precipitation is performed by both raising the salt concentration (typically to 0.5 mol L^{-1}) and adding a high molecular weight polyethylene glycol. Details vary among the different bacteriophages. The precipitation concentrates bacteriophage particles for subsequent steps in purification. Alternatively, bacteriophage particles can be concentrated by pelleting, without precipitation. The newer centrifuges can pellet bacteriophages in increased volume and decreased time. Bacteriophages in eight 50 mL tubes can now be pelleted in 1–4 h. The time depends on how rapidly the bacteriophage particles sediment at any given speed of centrifugation.

Subsequent Stages of Purification

Concentration and partial purification of bacteriophage particles are achieved in the first stage of purification. The subsequent stages can, in theory, be essentially any procedure of purification used for other biological macromolecules. However, in practice, the simplest, highest yielding, highest volume procedure has been found to be centrifugation. Three types of centrifugation are used, sometimes in tandem. The details of procedure vary with both the properties of the bacteriophage and the purposes of the investigator.

Buoyant Density Centrifugation

Buoyancy is a familiar concept to anyone who has learned to swim. Buoyancy can be used to purify any

macroscopic object by placing the object in solution that continuously varies in density. Gravity will drive the particle to the region of the density gradient that has a density equal to the density of the particle (isodense region). This concept also works for bacteriophages. However, bacteriophages are small. They are so small that ultracentrifugation is needed to drive them to an isodense region (a process called buoyant density centrifugation). Thermally driven motion (diffusion) randomizes the position of the bacteriophage particles in the absence of centrifugation. Even if diffusion did not occur, the bacteriophage particles would take impractically long to find their isodense region, if centrifugal force were not applied. Buoyant density centrifugation is performed by mixing bacteriophage particles with a solute that will form a density gradient when centrifuged ('before' column of the first row of **Figure 2**; the sample is grey). The density gradient forms because the solute, like the bacteriophage particles, is driven in the direction of the centrifugal force. The motion caused by centrifugal force eventually achieves equilibrium with thermal motion. The result is a density gradient. The bacteriophage particles are driven to an isodense region of the gradient ('after' column of the first row of Figure 2; the sample is black). Comparatively small ions (or molecules), like caesium cations, form the most linear density gradients. Caesium chloride is the most frequently used compound for fractionating bacteriophages by buoyant density centrifugation.

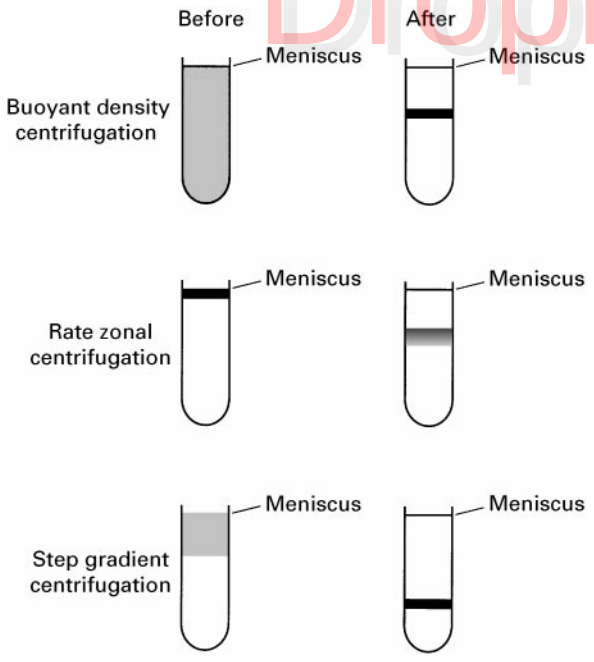

Figure 2 Purification of bacteriophage particles by ultracentrifugation. The three rows illustrate the three types of ultracentrifugation described in the text. The appearance of a centrifuge tube before centrifugation is shown in the column labelled before. The appearance of the same centrifuge tube after centrifugation is indicated in the column labelled after.

The word isodense means same density. But, what is the density of a bacteriophage particle? Can this density vary with position in a density gradient? This density not only can, but does, vary with position in a density gradient. The density varies because the anhydrous components of the bacteriophage are not the only components, from the perspective of buoyant density. The bacteriophage also contains both water- and density-forming solute. The concentration of water inside the bacteriophage is not necessarily the same as the concentration outside the bacteriophage. The bacteriophage nucleic acid sometimes binds a large amount of solute-free water, for example. But, this concentration does vary with the concentration of water outside of the bacteriophage. Analysis of this situation is beyond the scope of this article. This analysis is not critical for achieving practical results. However, it is critical for interpreting densities in terms of a particle's composition.

Many studied bacteriophages consist only of a protein capsid that contains a nucleic acid genome. The capsid has a density of roughly $1.3\ g\ mL^{-1}$ when centrifuged to an isodense region in a caesium chloride density gradient. Double-stranded DNA has a density of roughly $1.7\ g\ mL^{-1}$. Both these densities vary slightly with detailed composition. Double-stranded DNA bacteriophages are, in general, roughly 50% DNA, 50% protein. The density of these bacteriophages is $1.5\ g\ mL^{-1}$. This density is halfway between the density of protein and the density of DNA. This relationship is, however, not an intrinsic feature of densities. Its source is roughly equal hydration of DNA and protein in caesium chloride density gradients. These two hydrations are usually not equal.

Buoyant density centrifugation has the following limitation. Low molecular weight contaminants (molecules of RNA, unassembled proteins) diffuse so rapidly that they contaminate the bacteriophages, even though their density is different. Thus, bacteriophage purification procedures often have one stage at which particles are fractionated by size.

Rate Zonal Centrifugation

Rate zonal centrifugation fractionates particles by both size and shape. The procedure is to layer a sample in a restricted zone on top of a pre-poured density gradient. The density gradient is then centrifuged. All particles migrate into the density gradient, because the density gradient has only densities much lower than the densities of the particles being centrifuged (illustrated in the second row of **Figure 2**). The

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particles are fractionated primarily by size and shape. The larger a particle is, the more rapidly it sediments. The more spherically symmetrical a particle is, the more rapidly it sediments. Bacteriophages sediment much more rapidly than unassembled proteins and RNA. Thus, bacteriophages are separated from these particles by a single rate zonal centrifugation in a sucrose gradient. Some fragments of host cells co-sediment with bacteriophage particles. However, these fragments have a variable rate of sedimentation. The bacteriophage particles have a unique rate of sedimentation. Therefore, separation from most of these fragments occurs.

Rate zonal centrifugation has the following limitation. The sample occupies a small region of the centrifuge tube at the start of fractionation. Furthermore, the sample becomes less concentrated during fractionation. Thus, the amount of sample is more limited when compared to the amount fractionated by buoyant density centrifugation. During buoyant density centrifugation, the sample occupies the entire centrifuge tube at the start of fractionation. The sample becomes more concentrated during fractionation. Thus, rate zonal centrifugation is usually not the procedure of choice. However, some bacteriophages (including bacteriophage G) are not stable when centrifuged in caesium chloride density gradients. Rate zonal centrifugation is a reasonable alternative in this case. Unstable bacteriophages have been stabilized by including polyethylene glycol in the density gradient used for rate zonal centrifugation.

Combined Buoyant Density and Rate Zonal Centrifugation

Advantages of buoyant density centrifugation are combined with advantages of rate zonal centrifugation when the following hybrid procedure is used. A comparatively broad zone of sample is layered on a pre-formed caesium chloride density gradient (before column of the third row of Figure 2). The caesium chloride density gradient had been formed before centrifugation, by successfully layering 3–5 caesium chloride solutions. These solutions decrease in density, from bottom to top. The rapid diffusion of a caesium cations converts the discontinuous gradient (called a step gradient) into a more continuous gradient. The step gradient with layered sample is centrifuged until bacteriophage particles reach an isodense position in the gradient (after column of the third row of Figure 2). Thus, the advantages of buoyant density centrifugation are achieved: increase in the sample's concentration and fractionation by density. On the other hand, the advantage of rate zonal centrifugation is also achieved: separation of bacteriophage particles from rapidly diffusing components of the cell.

The advantages of a caesium chloride step gradients make them a method of choice for purifying many bacteriophages. A single centrifugation is sometimes sufficient to achieve the purity needed. Alternatively, a buoyant density centrifugation can be performed after centrifugation in a step gradient. Increase in concentration of the sample is achieved by combining bacteriophages from several step gradients in a single buoyant density gradient.

Nondenaturing Gel Electrophoresis

Bacteriophage particles can be fractionated by electrophoresis through gels. This procedure is called nondenaturing gel electrophoresis because the bacteriophage particles are intact (infective) during electrophoresis. In contrast, other procedures of electrophoresis are used to analyse the components of disassembled bacteriophage particles. The gels used for nondenaturing electrophoresis must have pore sizes large enough to admit bacteriophage particles (30–90 nm in radius). Polyacrylamide gels can be made dilute enough to achieve the needed pore sizes. However, these polyacrylamide gels are very weak and difficult to handle. Agarose gels achieve the needed pore sizes with gels that are easy to handle. Agarose has been the gel-forming compound most frequently used to fractionate bacteriophages (and other viruses) by nondenaturing gel electrophoresis.

Gel electrophoresis fractionates any spherical particle by two properties of the particle: the average electrical surface charge density (σ), and the radius. The value of σ is the sole determinant of the electrophoretic mobility (= velocity/electrical field) in the absence of the gel, within experimental accuracy. For example, a dimer of a bacteriophage capsid has an electrophoretic mobility indistinguishable from that of a capsid monomer, when the electrophoretic mobility is extrapolated to a gel concentration of zero. In theory, the value of σ will be the sole determinant of gel-free electrophoretic mobility, for all conditions likely to be encountered during the analysis of bacteriophage particles. Changing the value of the particle's radius will, however, change its electrophoretic mobility during electrophoresis through a gel. The larger the radius is, the more retarded the particle will be by the fibres that form the gel.

The fractionation by σ is independent of fractionations achieved by either buoyant density or rate zonal fractionation. Thus, nondenaturing gel electrophoresis can differentiate particles not differentiated by any procedure of centrifugation. The result has been the finding that at least one bacteriophage (T7) exists in more than one state. The different states appear to have evolved to improve the survivability of T7.

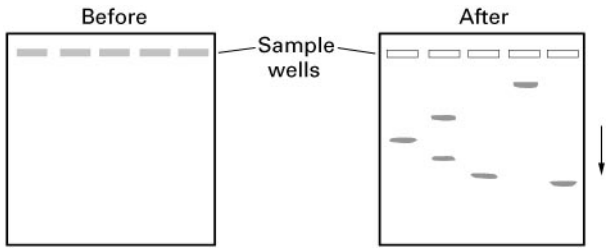

Figure 3 Nondenaturing gel electrophoresis. A horizontal gel with samples loaded is illustrated in the before panel. The same gel with stained, fractionated bacteriophages is shown in the after panel. The arrow indicates the direction of electrophoresis.

In practice, gel electrophoresis can be performed by the following procedure:

1. An agarose gel is cast in a horizontal orientation (before column in **Figure 3**). The gel has sample wells for placing the sample (indicated in Figure 3).
2. The gel is submerged beneath an electrophoresis buffer. The pH should be close to the pK_a of the buffer. The gel chosen should not adhere to the bacteriophage particles. Agarose can be purchased at several levels of purity. Several agarose derivatives and mixtures can be purchased. Some nonagarose polysaccharides can also be used for electrophoresis.
3. The sample is placed in a sample well. The sample contains an uncharged compound (glycerol, sucrose, a polyethylene glycol, for example), in order to prevent the sample from floating.
4. An electrical potential is placed across the gel. The electrical field and time of electrophoresis are chosen, based on the following:
   (a) The most rapidly migrating particle should not migrate out of the gel.
   (b) The separation between particles should be sufficient so that each particle forms a separate band.
   (c) Bacteriophage particles should not disrupt during electrophoresis.
   (d) The time of electrophoresis should be short enough so that band broadening is not a problem.
   (e) The electrical current should be low enough so that rise in temperature does not destabilize bacteriophage particles.

The bacteriophage particles are visualized by either light scattering or staining, after electrophoresis (illustrated in the after column of **Figure 3**). Ethidium is a stain used for DNA; Coomassie blue is a stain used for protein. Preparative fractionation should use light scattering, to avoid stain-induced changing of the bacteriophage particles.

Large scale fractionation of bacteriophage particles is almost never done by gel electrophoresis. Gel electrophoresis is used primarily for analysis, not purification. The reasons are the following:

1. Like rate zonal centrifugation, nondenaturing gel electrophoresis begins with the sample in a small volume. The sample is diluted during fractionation.
2. Recovery of particles after gel electrophoresis is not as good as recovery after fractionation in solution.
3. The bacteriophage particles are contaminated with both agarose and contaminants of agarose, after fractionation.
4. The bacteriophage particles are exposed to products of the electrolysis of water. The result is an unnecessary source of possible damage of bacteriophage particles.

Concluding Remarks

The purification of bacteriophages by centrifugation is a routine procedure. The most difficult requirement is the obtaining of centrifuges. Challenges are encountered primarily when a bacteriophage is unstable during exposure to the conventional conditions of centrifugation. A further challenge is encountered when bacteriophage particles are purified for the purpose of high resolution analysis of structure. This latter challenge is heterogeneity of bacteriophage particles that appear homogeneous when fractionated by centrifugation. Nondenaturing gel electrophoresis can reveal heterogeneity. However, nondenaturing gel electrophoresis has not been useful for preparative fractionation. Thus, a challenge for the future is the development of preparative procedures of nondenaturing gel electrophoresis. This last challenge would be met by development of a procedure of continuous-flow gel electrophoresis. Continuous-flow preparative gel electrophoresis is possible, in theory. Hopefully, it will be achieved in practice.

See also: **II/Electrophoresis:** Agarose Gels. **Centrifugation:** Theory of Centrifugation.

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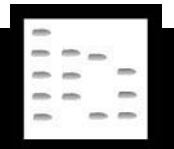
BALSAMS AND RESINS: THIN-LAYER (PLANAR) CHROMATOGRAPHY

See **III / ESSENTIAL OILS / Thin Layer (Planar) Chromatography**

BASES: THIN-LAYER (PLANAR) CHROMATOGRAPHY



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Introduction

According to the Brønsted–Lowry definition (1923) a base is a proton acceptor:

$$\underset{\text{base}}{B} + \underset{\text{proton}}{H^+} = \underset{\text{conjugated acid}}{BH^+}$$

The stronger the base, the larger is its K_b and consequently the smaller is its pK_b and the larger is the pK_a of the conjugated acid.

There are various types of bases, including natural and synthetic compounds, and they occur in a vast array of products, extending from the alkaloids, sulfa drugs (sulfonamides), dyes (azines, indoles), herbicides (simazine, atrazine, promazine), biogenic amines to numerous other groups.

This chapter includes only aliphatic and aromatic amines and their derivatives, heterocyclic bases and miscellaneous compounds (nitrosamines, amides, hydrazines). Thin-layer chromatography is used extensively for the analysis of bases and can achieve separations of complex mixtures comparable to column liquid chromatography.

Aliphatic Amines

The first attempts to separate aliphatic amines were performed on silica gel using chloroform–ammonia (39 : 1), chloroform–methanol–17% ammonia (2 : 2 : 1), butanol–acetic acid–water (4 : 1 : 5) and phenol–water (8 : 3) as eluents. However, highly volatile amines cannot be chromatographed with an ammoniacal solvent. A systematic collection of data on the chromatographic behaviour of a large number of aliphatic amine hydrochlorides, with particular emphasis on eluents, adsorbents and detection reagents, was published by Prandi (see hR_F values in **Table 1**, columns 1, 2 and 3).

Silica gel is particularly useful for the adsorption chromatography of amines that have different polarities but does not resolve the fatty amine series. In particular, the R_F values increase as the aliphatic chain length increases but this increase becomes smaller with increasing chain length.

Reversed-phase partition chromatography on paraffin oil–saturated silica gel is useful for the separation of fatty amines. $R_M[\log(1/R_F)^{-1}]$ values for such amines increase as the length of the aliphatic chain increases and there is a linear relationship between R_M and the number of carbon atoms in the molecule.